Biomarkers for Tobacco Use During Nicotine Replacement Therapy

- **NEEDED TO VALIDATE CESSATION OF TOBACCO USE**
- **NEEDED TO MEASURE TOXIC SUBSTANCE EXPOSURE IN HARM REDUCTION STUDIES**
- **MUST BE DERIVED FROM TOBACCO, BUT NOT PRODUCED BY METABOLISM OF NICOTINE**
- **NO SIGNIFICANT ENVIRONMENTAL OR DIETARY SOURCES**
- **ANALYTICAL METHOD SUITABLE FOR ANALYSIS OF LARGE NUMBER OF SAMPLES GENERATED IN CLINICAL STUDIES**

Biomarkers and Toxic Substances in Tobacco Smoke

Tobacco Smoke Component	µg/Cigarette
Carbon Monoxide	2000-15,000
Nicotine	800-3000
Hydrogen Cyanide	100-400
Solanesol	10-500
Anatabine	3.7-14
Anabasine	3-12
N'-Nitrosonornicotine (NNN)	0.029-3.7
4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)	0.013-0.47
Pyrene	0.020-0.100
Benzo[a]pyrene	0.008-0.053
4-Aminobiphenyl	0.0002-0.023

Wynder, E.L., and Hoffmann, D. (1967) *Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis.* Academic Press, NY.
Schmeltz and Hoffmann (1977) Chem. Rev, 295-311.
Hoffmann, D., Djordjevic, M.V., Hoffmann, I. (1997) Preventive Medicine, 26:427-434.

Characteristics of a Valid Biomarker

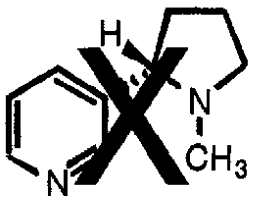
- **SHOULD BE UNIQUE OR NEARLY UNIQUE TO TOBACCO SO THAT OTHER SOURCES ARE MINOR IN COMPARISON (SPECIFICITY ADEQUATE TO MINIMIZE FALSE POSITIVES)**
- **SHOULD BE EASILY DETECTABLE (SENSITIVITY ADEQUATE TO MINIMIZE FALSE NEGATIVES)**
- **SHOULD BE PRODUCED IN SIMILAR AMOUNTS FOR A VARIETY OF TOBACCO PRODUCTS**
- **SHOULD HAVE A FAIRLY CONSTANT RATIO TO OTHER COMPONENTS OF INTEREST UNDER A RANGE OF SMOKING CONDITIONS**

National Research Council (1986). *Environmental Tobacco Smoke. Measuring Exposures and Assessing Health Effects.* National Academy Press, Washington, D.C.

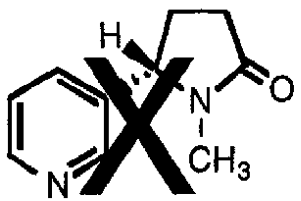
Important Characteristics of a Biomarker

- **BIOLOGICAL HALF-LIFE**
- **INDIVIDUAL VARIABILITY IN METABOLISM**
- **AVAILABILITY OF AN ANALYTICAL METHOD WITH ADEQUATE SENSITIVITY, PRECISION, AND ACCURACY**

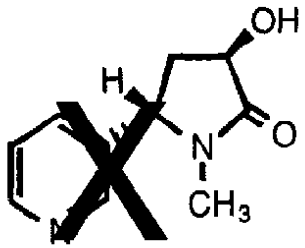
Tobacco Biomarkers and Potential Biomarkers



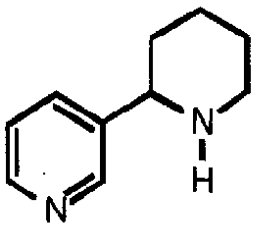
NICOTINE



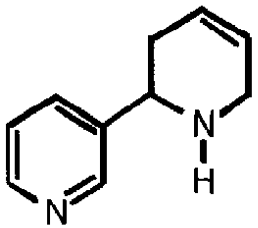
COTININE



TRANS-3'-HYDROXYCOTININE



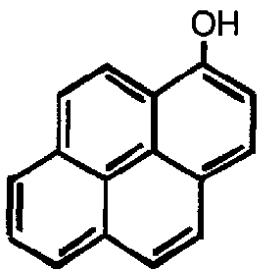
ANABASINE



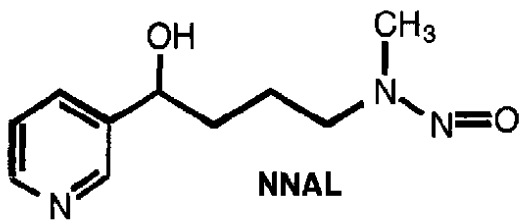
ANATABINE

CARBON MONOXIDE

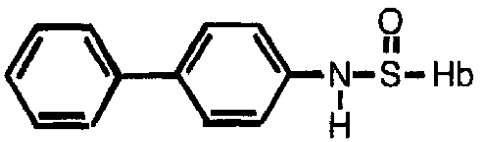
THIOCYANATE



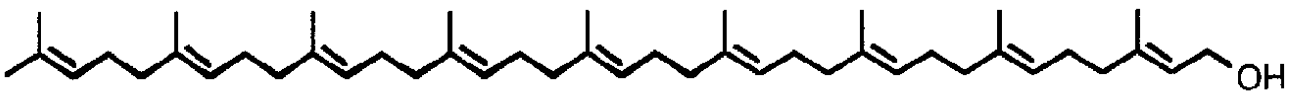
1-HYDROXYPYRENE



NNAL



4-AMINOBIPHENYL HEMOGLOBIN ADDUCT



SOLANESOL

Carbon Monoxide

C≡O

SOURCE: COMBUSTION PRODUCT

TYPICAL LEVELS:

	SMOKER	NON-SMOKER
EXPIRED AIR (PPM)	20.8	5.7
BLOOD CARBOXYHEMOGLOBIN (%)	3.9	0.94

BIOLOGIC HALF-LIFE: 3-4 HOURS (SEDENTARY ACTIVITY) 5-8 HOURS (DURING SLEEP)

ANALYTICAL METHODS: ELECTROCHEMICAL (ECOLYZER) FOR BREATH
SPECTROPHOTOMETRIC (CO-OXIMETER) FOR BLOOD

APPLICATIONS: VALIDATE SMOKING CESSATION - DETECT RECENT SMOKING
CARBOXYHEMOGLOBIN CORRELATION WITH NICOTINE INTAKE: R = 0.69 (4:00 PM)

ADVANTAGES: SIMPLE AND FAST FOR EXPIRED CO

DISADVANTAGE: ENVIRONMENTAL SOURCES, SHORT AND VARIABLE HALF-LIFE

Jarvis et al. (1984) *J. Epidemiol. Commun. Health* 38:335-39
Benowitz (1984) NIDA Monograph # 48
Benowitz and Jacob (1984) *Clin. Pharmacol. Ther.* 35:499-504

Thiocyanate

$[S-C\equiv N]^-$

SOURCE: HYDROGEN CYANIDE FROM COMBUSTION METABOLIZED TO THIOCYANATE

TYPICAL LEVELS:

	SMOKER	NON-SMOKER
PLASMA (μM)	158	33
SALIVA (μM)	3340	1290

BIOLOGIC HALF-LIFE: 14 DAYS

ANALYTICAL METHODS: SPECTROPHOTOMETRIC, GC, ION CHROMATOGRAPHY

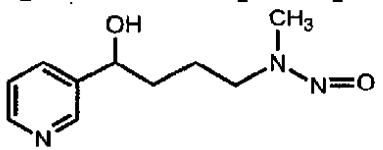
APPLICATIONS: VALIDATE SMOKING CESSATION, ESTIMATE TOBACCO SMOKE EXPOSURE

ADVANTAGES: LONG HALF-LIFE, RELATIVELY SIMPLE ASSAY

DISADVANTAGE: DIETARY SOURCES

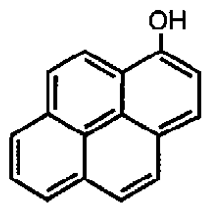
Benowitz (1984) NIDA Monograph #48
Jacob et al. (1984) Analyt. Chem. 56:1692-1695
Haley et al. (1983) Am. J. Public Health 73:1204-1205

4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanol (NNAL)



SOURCE:	**METABOLITE OF TOBACCO-SPECIFIC NITROSAMINE NNK**		
TYPICAL LEVELS:		**SMOKER**	**NON-SMOKER**
(PLUS GLUCURONIDE)	**EXCRETION IN URINE (NG/DAY)**	**3200**	**42**
BIOLOGIC HALF-LIFE:	**40-50 DAYS**		
ANALYTICAL METHODS:	**GC-THERMAL ENERGY ANALYZER, GC-MS, LC-MS/MS**		
APPLICATIONS:	**CARCINOGEN EXPOSURE** **CORRELATION WITH URINE COTININE CONCENTRATION R= 0.58**		
ADVANTAGES:	**METABOLITE OF POTENT LUNG CARCINOGEN**		
DISADVANTAGE:	**VERY LONG HALF-LIFE**		

Hecht and Tricker (1999) In "Analytical Determination of Nicotine and Related Compounds and Their Metabolites," Gorrod and Jacob, eds, Elsevier, pp 421-499.
Carmetta et al. (1995) Cancer Epidemiol. Biomarkers & Prev. 4:635-642



1-Hydroxypyrene (1-HP)

SOURCE: PYRENE FORMED DURING COMBUSTION IS METABOLIZED TO 1-HP

TYPICAL LEVELS:

	SMOKER	NON-SMOKER
URINE (µMOL/MOL CREATININE)	0.25	0.12

BIOLOGIC HALF-LIFE: 10 HOURS

ANALYTICAL METHODS: HPLC-FLUORESCENCE

APPLICATIONS: CARCINOGEN EXPOSURE

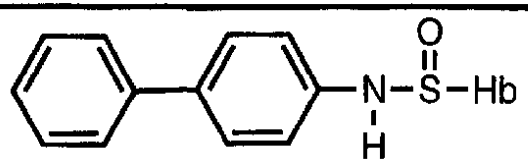
CORRELATION WITH CIGARETTES PER DAY: R = 0.67 (JOOST ET AL)
R = 0.34 (VINEIS ET AL.)

ADVANTAGES: MARKER FOR PAH EXPOSURE

DISADVANTAGE: ENVIRONMENTAL AND DIETARY SOURCES

Joost et al. (1994) Int. Arch. Occup. Environ Health 66:55-65
Vineis et al. (1996) Int. J. Cancer 65:314-316

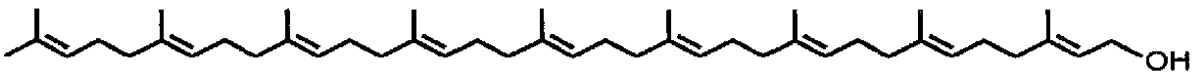
4-Aminobiphenyl Hemoglobin Adducts



SOURCE:	**COVALENT BINDING OF N-OXIDATION PRODUCT**		
TYPICAL LEVELS:		**SMOKER**	**NON-SMOKER**
	BLOOD (PG/G OF HEMOGLOBIN)	**154**	**28**
BIOLOGIC HALF-LIFE:	**~60 DAYS**		
ANALYTICAL METHODS:	**GC-MS WITH NICI**		
APPLICATIONS:	**CARCINOGEN EXPOSURE**		
	CORRELATION WITH URINE COTININE CONCENTRATION		**R= 0.57**
	CORRELATION WITH CIGARETTE CONSUMPTION		**R= 0.75**
ADVANTAGES:	**METABOLITE OF BLADDER CARCINOGEN**		
DISADVANTAGE:	**ENVIRONMENTAL SOURCES, LONG HALF-LIFE**		

Vineis et al. (1996) Int. J. Cancer 65:314-316
Bryant et al (1987) Cancer Research 47:602-608
Hammond et al. (1993) JNCI 85:474-478

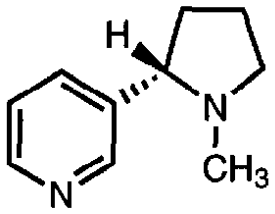
New Biomarker Being Developed – Solanesol



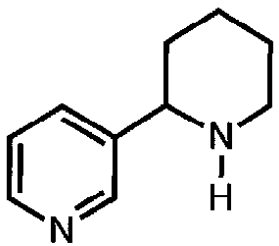
SOURCE:	**MAJOR COMPONENT OF TOBACCO SMOKE**		
TYPICAL LEVELS:		**SMOKER**	**NON-SMOKER**
	SALIVA (NG/ML)	**45**	**0.6**
BIOLOGIC HALF-LIFE:	**UNKNOWN**		
ANALYTICAL METHOD:	**LC-MS/MS**		
APPLICATIONS:	**TOBACCO SMOKE PARTICULATE ("TAR") MARKER**		
ADVANTAGES:	**MAJOR COMPONENT OF PARTICULATE PHASE OF TOBACCO SMOKE** **NEUTRAL LIPOPHILIC SUBSTANCE · GOOD BIOMARKER FOR PAH?** **USED AS A TRACER FOR ETS:** **EXTENSIVE LITERATURE ON LEVELS IN TOBACCO SMOKE**		
DISADVANTAGE:	**ADDITIONAL STUDIES NEEDED**		

Jacob et al. (2001) Society for Research on Nicotine and Tobacco, Annual Meeting, Seattle

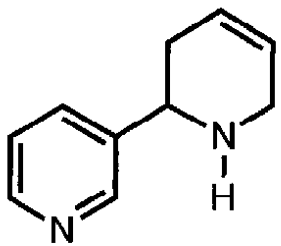
ANABASINE AND ANATABINE AS BIOMARKERS DURING NRT



NICOTINE



ANABASINE



ANATABINE

- **MINOR TOBACCO ALKALOIDS STRUCTURALLY RELATED TO NICOTINE NOT PRESENT IN PHARMACEUTICAL PRODUCTS (US)**
- **NO SIGNIFICANT DIETARY OR ENVIRONMENTAL SOURCES PRESENT IN URINE OF SMOKERS AND SMOKELESS TOBACCO USERS PROPOSED AS BIOMARKERS FOR TOBACCO USE DURING NRT**

Relative Molar Potency of Nicotine and Other Cigarette Smoke Alkaloids

Alkaloid	Contraction of Guinea-Pig Ileum	Pressor Action in Pithed Rat	Release of Catechol-amines From Cat Adrenal	Inhibition of Cat Knee Jerk	Inhibition of Chick Flexor Reflex
Nicotine	100	100	100	100	100
Nornicotine	4.5	22	55	54	36
Anabasine	17.5	20	75	17	33
Myosmine	0.2	5.5	–	3	3
Nicotyrine	0.3	2.5	–	17	51
2:3-Dipyridyl	0.2	–	–	<0.1	–
Cotinine	<0.001	<0.1	0.03	<0.05	–

Clark MSG, Rand MJ, Vanov S. Comparison of pharmacological activity of nicotine and related alkaloids occurring in cigarette smoke. Arch. Int. Pharmacodyn. 156:363-379, 1965.

CLINICAL PHARMACOLOGY OF ANABASINE AND ANATABINE

- NOTHING KNOWN ABOUT PHARMACOLOGY OF ANATABINE
- HALF LIVES IN HUMANS BASED ON URINARY EXCRETION DATA:

  ANABASINE, 16 HOURS
  ANATABINE, 10 HOURS

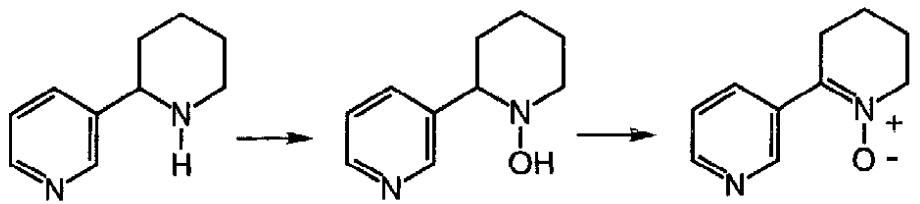
  Jacob et al. (1999) Am. J. Public Health 89:731-736

- ANABASINE TABLETS HAVE BEEN USED TO AID SMOKING CESSATION IN RUSSIA

METABOLISM OF ANABASINE AND ANATABINE

Biotransformation

- **NOTHING PUBLISHED ON METABOLISM OF ANATABINE**
- **ANABASINE CONVERTED TO N'HYDROXYANABASINE AND THE CORRESPONDING NITRONE IN VITRO:**



Becket et al. (1973) J. Pharm. Pharmacol. 25:171P

CONCENTRATIONS OF TOBACCO ALKALOIDS IN VARIOUS TOBACCO PRODUCTS

Alkaloid	Type of Tobacco				
	Cigarette (13 Brands)	Oral Snuff (4 Brands)	Chewing (3 Brands)	Pipe (3 Brands)	Cigar (5 Brands)
Nicotine mg/g (SD)	17.5 (2.20)	13.5 (4.07)	6.49 (3.27)	14.4 (2.10)	9.13 (0.822)
Nicotine % of total	96.2%	97.9%	96.5%	97.0%	91.9%
Nornicotine mg/g (SD)	0.382 (0.071)	0.173 (0.034)	0.140 (0.016)	0.199 (0.044)	0.658 (0.4)
Nornicotine % of total	2.11%	1.32%	2.35%	1.37%	6.54%
Anabasine mg/g (SD)	0.030 (0.0039)	0.017 (0.0025)	0.0085 (0.0020)	0.029 (0.0081)	0.029 (0.0030)
Anabasine % of total	0.16%	0.13%	0.14%	0.20%	0.29%
Anatabine mg/g (SD)	0.271 (0.034)	0.084 (0.024)	0.0650 (0.024)	0.214 (0.068)	0.127 (0.036)
Anatabine % of total	1.49%	0.62%	1.01%	1.48%	1.28%

Jacob et al. (1999) Am. J. Public Health 89:731-736

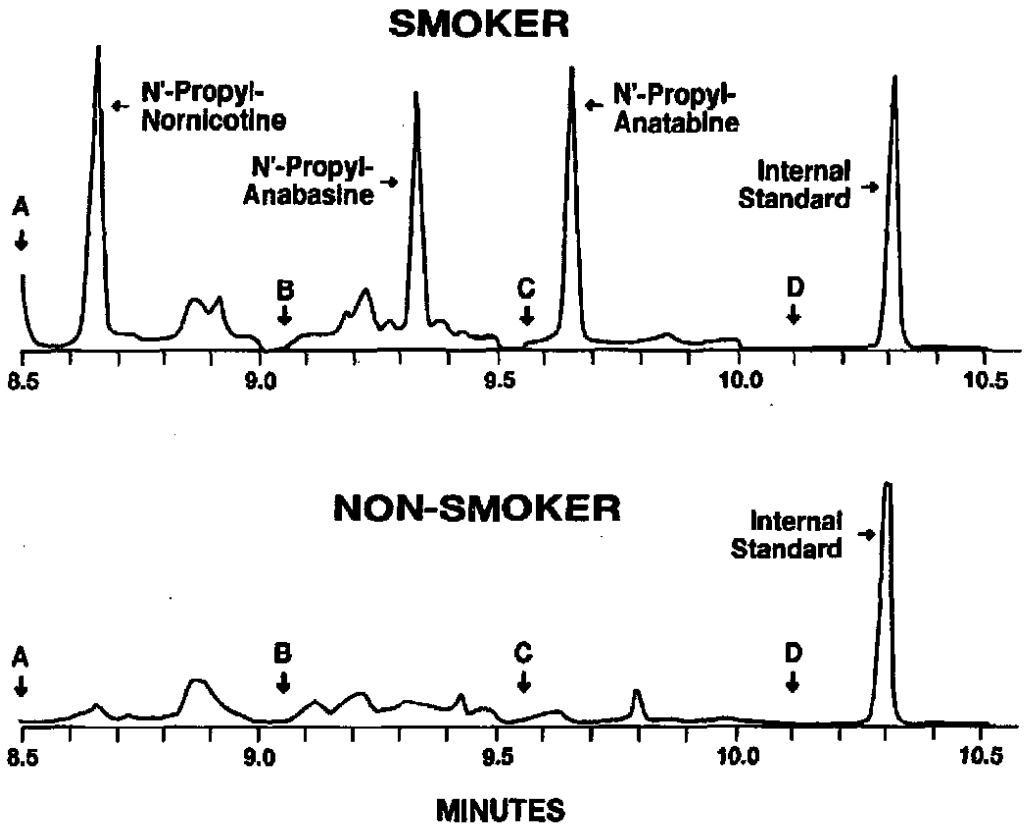
DETERMINATION OF ANABASINE AND ANATABINE IN URINE USING GC-MS

1. **Pipet 5 mL urine, add internal standard**
2. **Solid-phase extraction**
   Wash with water, elute with methanol
3. **Evaporate eluate**
4. **Derivatize with propionaldehyde/$NaBH_4$**
5. **Add aqueous NaOH**
   Extract with toluene/butanol (70:30)
6. **Separate organic layer**
7. **Back-extract into aqueous H_2SO_4**
8. **Separate aqueous layer**
   Make basic with aqueous K_2CO_3
9. **Extract with toluene-butanol (90:10)**
   Concentrate, inject into GC-MS

LIMIT OF QUANTITATION: 1 ng/mL

Jacob et al. (1993) J. Chromatogr. 619:49-61.

GC-MS Ion Chromatograms of Urine Extracts



A - B, m/z 161; B - C, m/z 175; C - D, m/z 173; D m/z 189

MEAN CONCENTRATIONS OF TOBACCO ALKALOIDS IN URINE OF SMOKERS AND SMOKELESS TOBACCO USERS

	n		Anabasine	Anatabine	Nicotine	Cotinine
Smokeless Tobacco Study 1	100	ng/mL (SD)	24 (31)	41 (51) [b]	1310 (1170)	2420 (1730)
		Range	0-201	0-246	0-4780	264-9470
Smokeless Tobacco Study 2	105	ng/mL (SD)	23 (30)	45 (61) [b]	1550 (1650)	2310 (1300)
		Range	0-208	0-456	10-8320	254-5920
Cigarette Smokers Study 3	99	ng/mL (SD)	22 (23)	22 (24) [b]	1960 (1770)	1790 (1030)
		Range	0-120	0-118	9.2-7940	187-4980

Urine concentrations prior to beginning tobacco cessation programs.

[a] $P < .05$ comparing smokeless tobacco versus cigarettes

[b] $P < .005$ comparing smokeless tobacco versus cigarettes

Jacob et al. Submitted, 2001, Am. J. Public Health

SPECIFICITY OF ANABASINE AND ANATABINE FOR TOBACCO USE

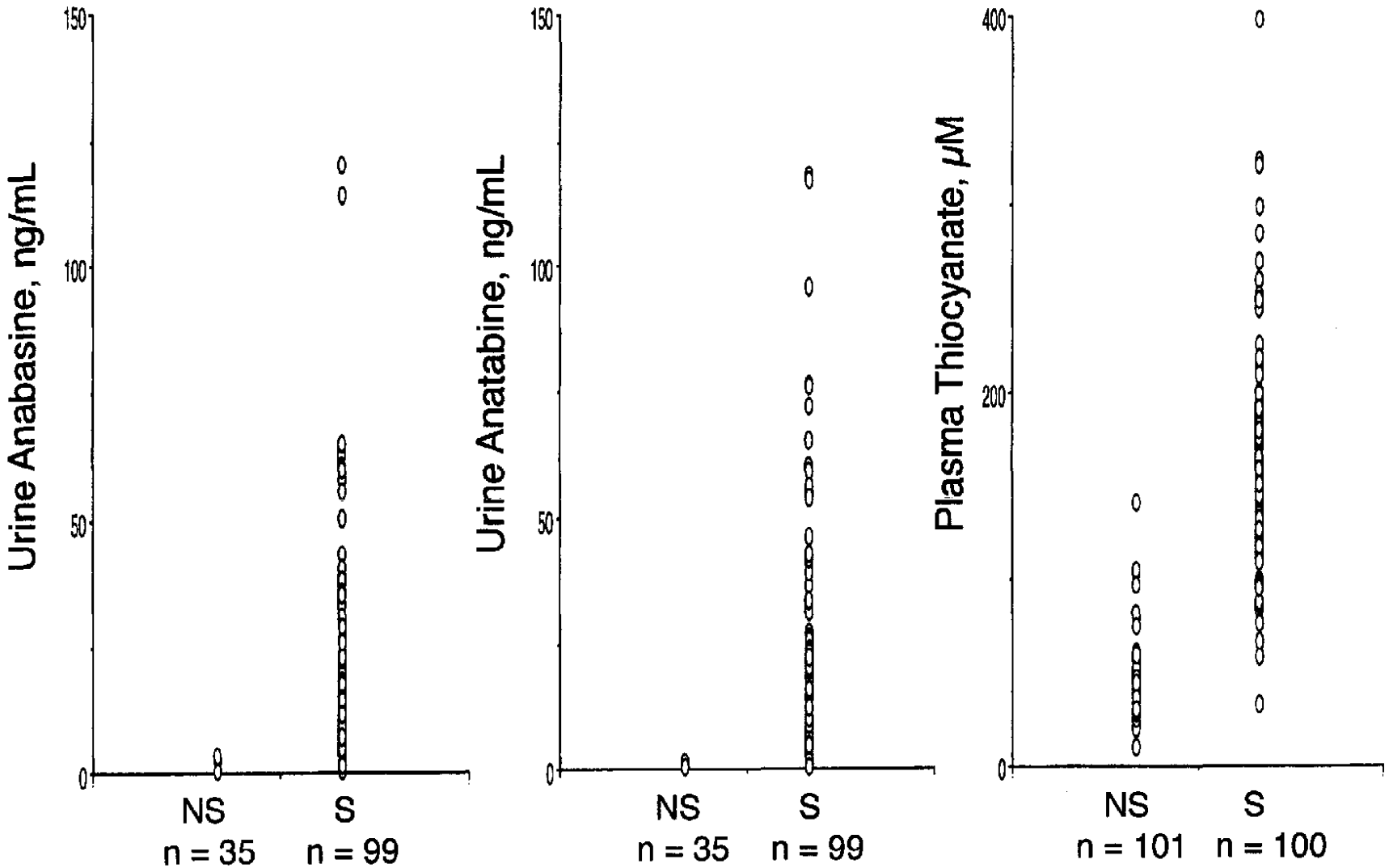
Study	N	Anabasine ng/ml	Anatabine ng/ml	Nicotine ng/ml	Cotinine ng/ml
Cigarette Smokers[1] mean (range)	99	22 (0-120)	22 (0-118)	1960 (9.2-2940)	1790 (187-4980)
Nonsmokers mean (range)	35	0.27 (0-3.4)	0.09 (0-1.8)	—	*
Abstinent Smokeless Tobacco Users on Nicotine Gum Therapy[2] mean (range)	19	0.37 (0-2.9)	0.18 (0-1.7)	376 (0-1790)	565 (110-1250)
Abstinent Smokeless Tobacco Users on Nicotine Gum Therapy[3] mean (range)	118	<1	<1		

* Plasma cotinine <10 ng/ml

1. Prior to beginning smoking cessation study directed by Sharon Hall, Ph.D.
2. From smokeless tobacco cessation study directed by Herb Severson, Ph.D.
3. From smokeless tobacco cessation study directed by Dorothy Hatsukami, Ph.D.

Using cut-point of 2 ng/mL for both anabasine and anatabine, specificity for tobacco use is 100%

Specificity of Anabasine and Anatabine for Tobacco Use: Comparison with Thiocyanate



Anabasine and Anatabine Determined by GC-MS: Jacob et al. (1993) J. Chromatography 619:49-61.
Thiocyanate Determined by Gas Chromatography: Jacob et al. (1984) Analytical Chemistry, 56: 1692-1695

URINE ANABASINE AND ANATABINE CONCENTRATIONS AS OUTCOME MEASURES IN SMOKELESS TOBACCO CESSATION STUDIES EMPLOYING NICOTINE GUM[a]

	Study 1	Study 2
Number of Subjects Completing Study	76	103
Number Claiming Abstinence	45	89
Validated Abstinence	45 (100%)	70 (79%)
Number of Deceivers[b]	0 (0%)	19 (21%)
Number Reporting Relapse	31	14
Number of False Negatives[c]	7 (23%)	5 (36%)

[a] Subjects were considered to be using tobacco if concentrations of both anabasine and anatabine in urine were above 2 ng/mL.

[b] Deceivers are defined as those who claim abstinence but are judged to be using tobacco based on urine anabasine and anatabine levels.

[c] False negatives defined as those who report relapse to tobacco use, but whose urine anabasine and anatabine levels are below those set to define tobacco use.

Jacob et al. Submitted, 2001, Am. J. Public Health

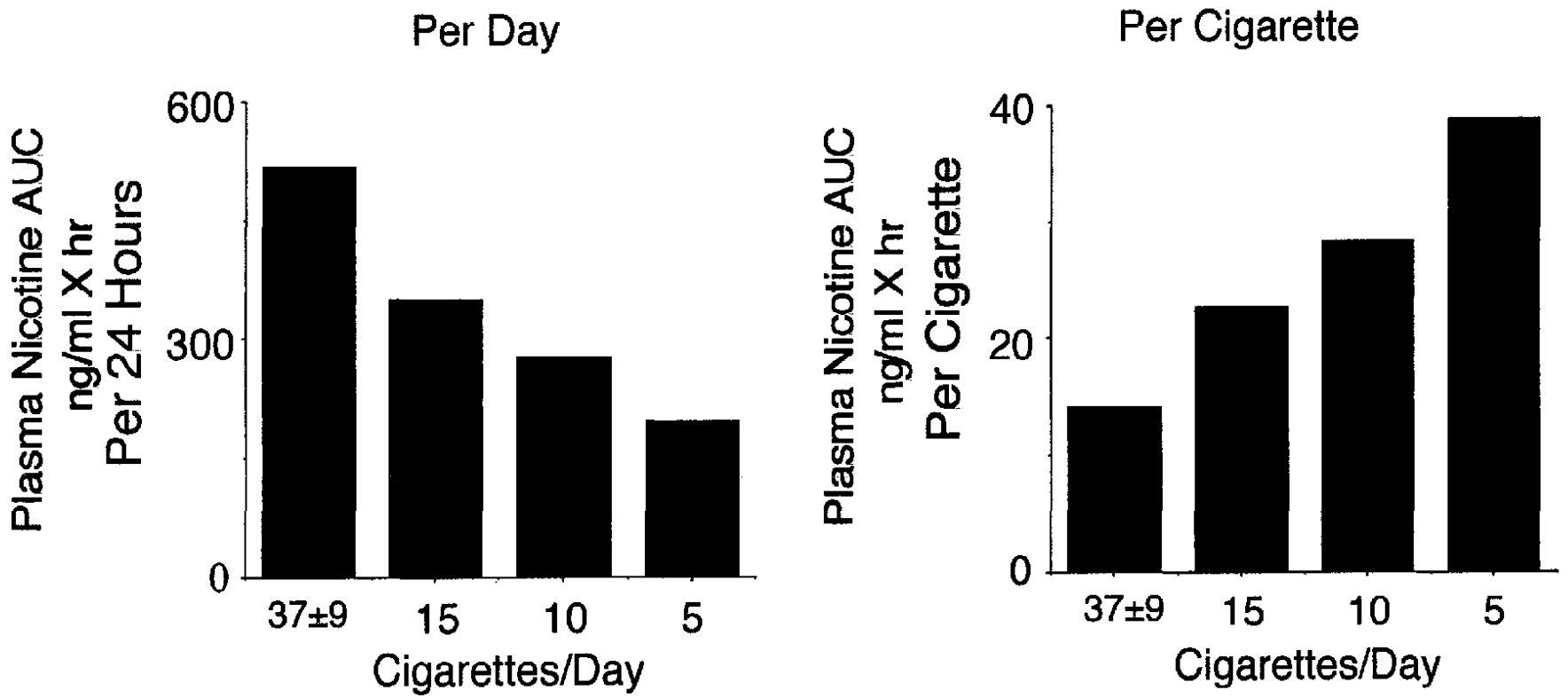
DETERMINATION OF ANABASINE AND ANATABINE IN URINE USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS)

1. **Pipet 1 mL urine, add internal standard, anabasine-d_4**
2. **Make basic with aqueous K_2CO_3**
3. **Extract with organic solvent**
4. **Evaporate extract**
5. **Reconstitute in HPLC mobile phase**
6. **Inject into LC-MS/MS**

LIMIT OF QUANTITATION: 0.2 ng/mL for anabasine
0.1 ng/mL for anatabine

Jacob, Yu, Benowitz. Manuscript in preparation

Why Biomarkers are Needed for Harm Reduction Studies: Influence of Smoking Fewer Cigarettes on Nicotine Exposure



Factor of 7.4 Reduction in Number of Cigarettes Smoked
Factor of 2.7 Reduction in Daily Nicotine Exposure
Factor of 2.7 Increase in Nicotine Exposure per Cigarette

Benowitz et al. (1986) N Engl J Med, 315:1310-3.

Validation of Biomarkers

CORRELATE BIOMARKER CONCENTRATION WITH SELF-REPORT

PLASMA COTININE WITH CIGARETTES PER DAY: R = 0.40
(Benowitz et al., 1983)

CORRELATE CONCENTRATIONS OF TWO BIOMARKERS OR OTHER MEASURE OF TOXIC SUBSTANCE EXPOSURE

URINE COTININE WITH NNAL: R = 0.58
(Carmella et al., 1995)

USE NICOTINE INTAKE FROM TOBACCO AS MEASURE OF TOBACCO SMOKE EXPOSURE. CORRELATE WITH BIOMARKER CONCENTRATION
(Benowitz and Jacob, 1984, 1994)

PLASMA COTININE WITH NICOTINE INTAKE: R = 0.82
(Benowitz and Jacob, 1984)

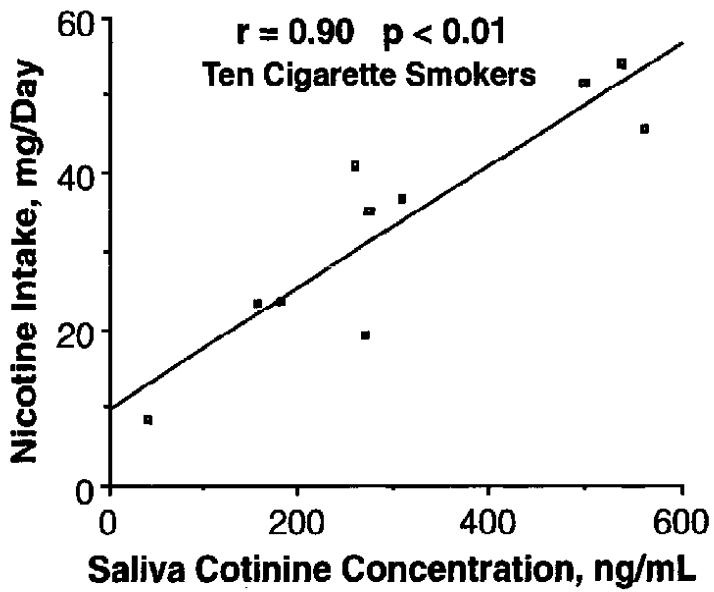
Benowitz et al., (1983) New Engl. J. Med. 309: 139-142
Carmetta et al. (1995) Cancer Epidemiol. Biomarkers & Prev. 4:635-642
Benowitz and Jacob (1984) Clin. Pharmacol. Ther. 35:499-504
Benowitz and Jacob (1994) Clin. Pharmacol. Ther. 56:584-593

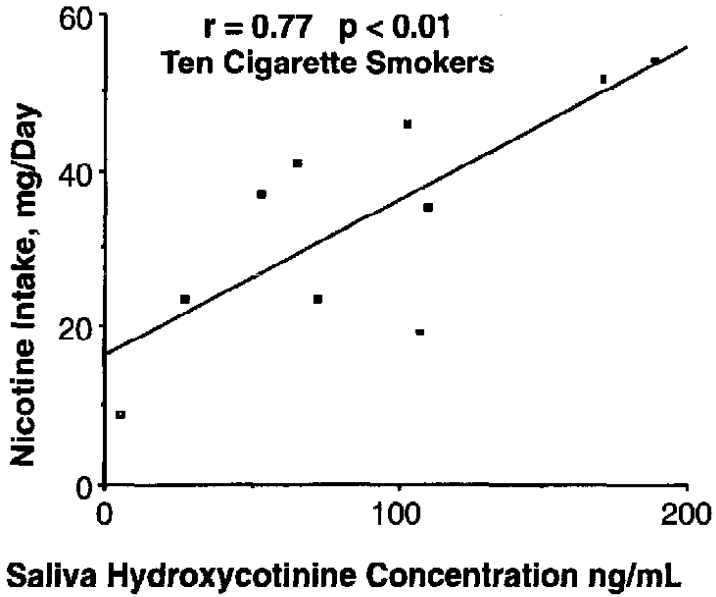
Nicotine Intake — Biomarker Correlations

DETERMINE NICOTINE INTAKE USING PHARMACOKINETIC TECHNIQUES ANALOGOUS TO DRUG BIOAVAILABILITY STUDIES

NICOTINE CLEARANCE = I.V. DOSE NICOTINE-D_2 / AUC NICOTINE-D_2
TOBACCO NICOTINE INTAKE = NICOTINE CLEARANCE X AUC OF NATURAL NICOTINE

CORRELATE NICOTINE INTAKE WITH BIOMARKER CONCENTRATION:



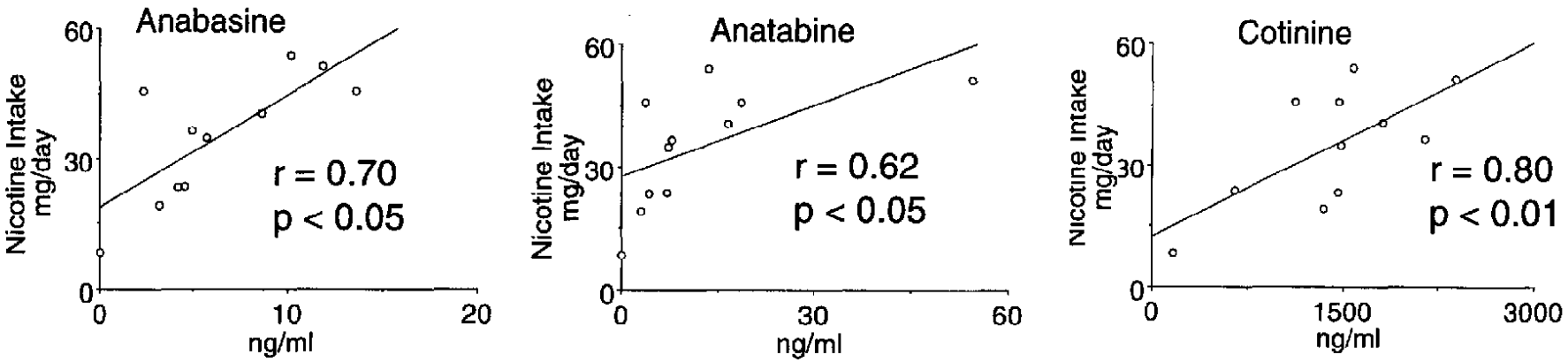


ADVANTAGE: CONTROLS FOR INDIVIDUAL DIFFERENCES IN METABOLISM
DISADVANTAGE: REQUIRES HOSPITAL SETTING AND INTRAVENOUS INFUSION

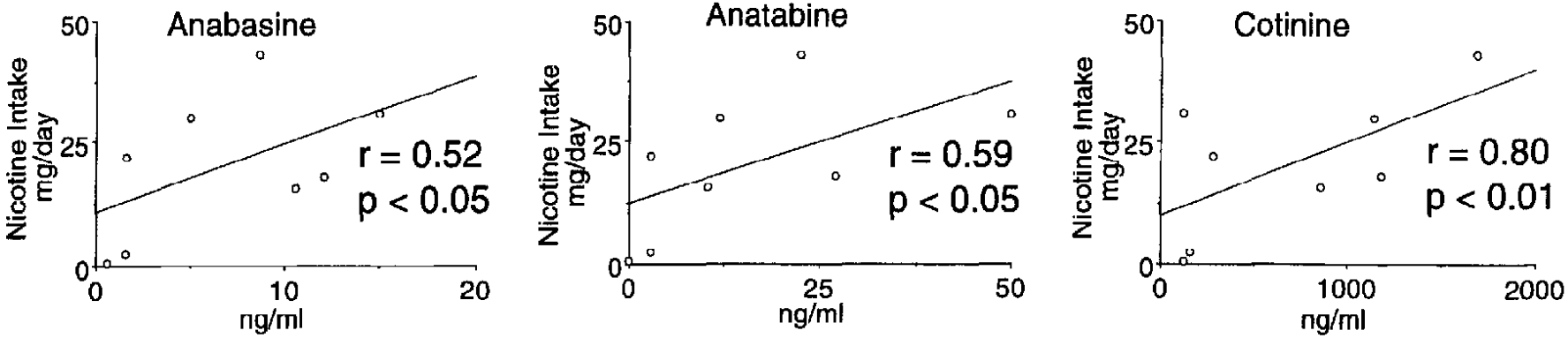
Benowitz et al. (1991) Clin. Pharmacol. Ther. 49:270-277
Jacob, Benowitz, Yu, unpublished data

Anabasine and Anatabine for Tobacco Use During Nicotine Replacement Therapy
Correlation of Biomarker Concentrations in Urine with Nicotine Intake

Cigarette Smokers (n = 12)

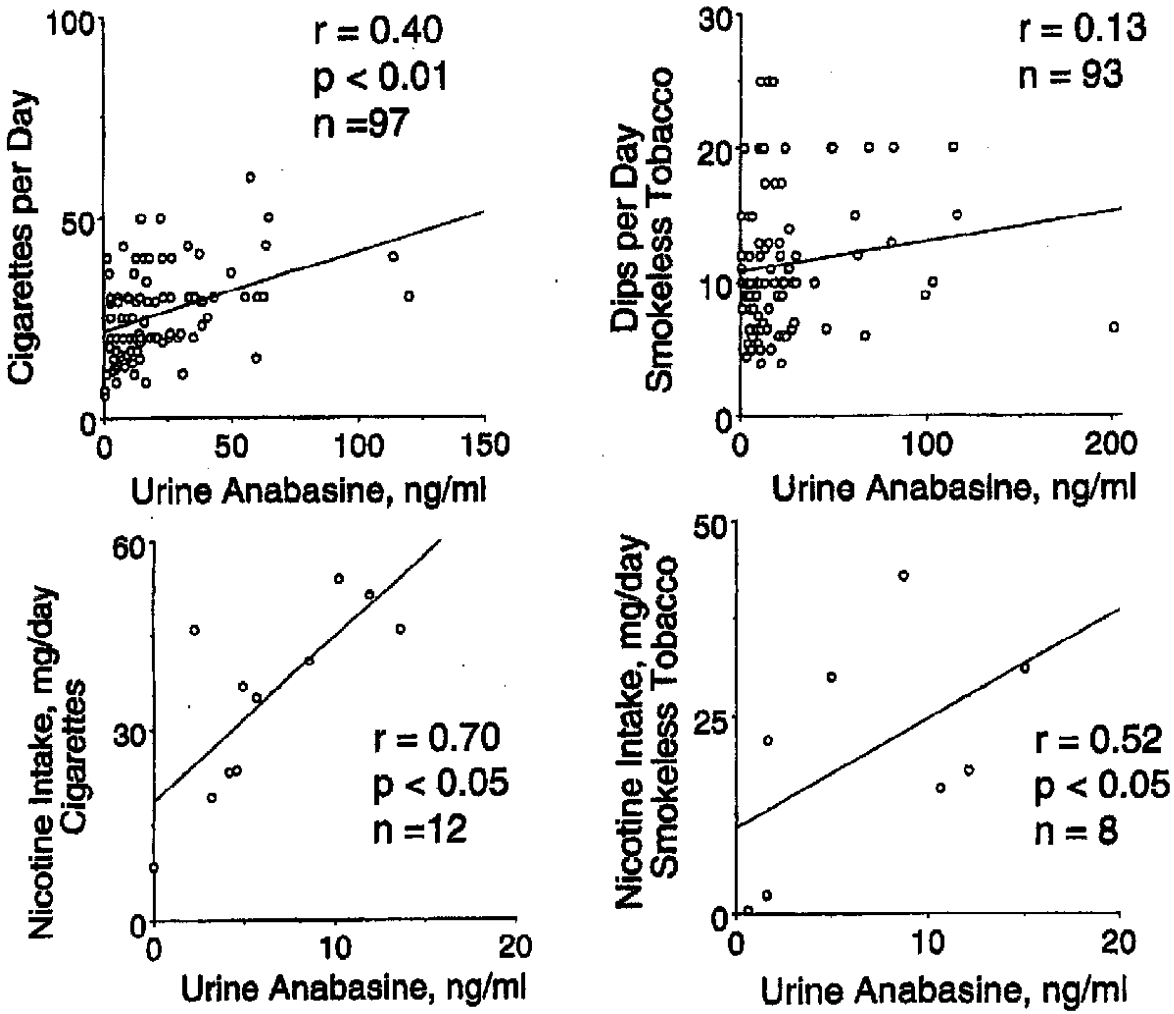


Smokeless Tobacco Users (n = 8)



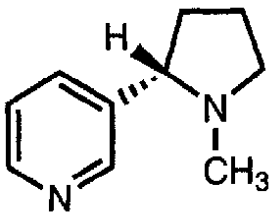
Jacob et al. (1999) Am. J. Public Health 89:731-736

Why Not Use Self-Report?

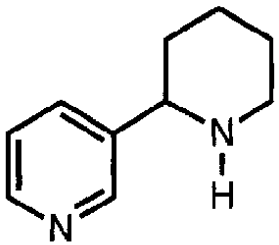


Should use Duration vs. frequency measures to correlate

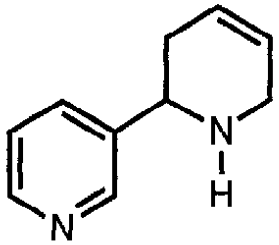
Jacob et al. Submitted, 2001, Am. J. Public Health



NICOTINE



ANABASINE



ANATABINE

- **Anabasine and anatabine are minor tobacco alkaloids that are structurally related to nicotine.**
- **They are not present in NRT medications, and do not seem to have significant dietary or environmental sources.**
- **Specificity for tobacco is excellent and, therefore, anabasine and anatabine are highly suitable for validating cessation of tobacco use during NRT.**
- **Since urine concentrations correlate well with measures of tobacco consumption, such as nicotine intake, they can be used to evaluate potential harm reduction in persons who cut down but are unable to quit.**

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Notes: